FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **214038US0PCT**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 09/926234

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/02268	**29 MARCH 1999**	**NONE**

TITLE OF INVENTION

PEPTIDES USEFUL IN TREATING MULITPLE SCHEROSIS AND A PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

APPLICANT(S) FOR DO/EO/US

Maria MARINO, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Request for Consideration of Documents in International Search Report

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/926234	PCT/EP99/02268	214038US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	3 - 20 =	0	x $18.00	**$0.00**	
Independent claims	3 - 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$990.00**	
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$990.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$990.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$990.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

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Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

9-28-01
DATE

Docket No.: 214038US0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Maria MARINO, et al. : ATTN: BOX SEQUENCE

SERIAL NO.: 09/926,234 :

FILED: OCTOBER 22, 2001 :

FOR: PEPTIDES USEFUL IN TREATING MULTIPLE SCLEROSIS AND A PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

PRELIMINARY AMENDMENT AND STATEMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Office Communication mailed October 29, 2001, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please delete the original Sequence Listing.

Page 14, after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.

REMARKS

Claims 1-3 are pending in the present application.

Applicants have now submitted an substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the

corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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PEPTIDES USEFUL IN TREATING MULTIPLE SCLEROSIS AND A PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

This invention relates to a peptide compound useful in treating the Multiple Sclerosis and a pharmaceutical composition comprising the same.

Multiple Sclerosis (MS) is the most common acquired demyelinating disease of the central nervous system. In particular, MS is characterized pathologically by plaques or islands of demyelination disseminated through the Central Nervous System (CNS), primarily in the white matter, the optic nerves, and periventricular areas.

In fact the loss of myelin, which wraps up neurons of CNS, is accompanied by a disruption in the ability of the nerves to conduct electrical impulses to and from the brain, and this produces the various symptoms of MS. Such symptoms depend on which areas of the central nervous system have been affected. The systems commonly affected include the vision, co-ordination, sensation, cognitive functions, bladder control, speech with slow enunciation with a tendency to hesitate at the beginning of a word or syllable, but also paresthesias in one or more extremities, in the trunk, or on one side of the face, weakness or clumsiness of a leg or a hand.

The course is highly varied and unpredictable and in most patients, remittent. Some people are minimally affected by the disease while others have rapid progress to total disability.

In addition, it is known that women are more likely to develop MS than men and the disease is more common in temperate climates (1:2000) than in the tropics (1:10,000).

Presently a therapy capable of stopping the progression of primary neurologic disability caused by MS is not known. Current therapy favors corticosteroids, which accelerate recovery from acute attacks, but are

not believed to alter the long-term course. Immunosuppressants, as cyclophosphamide, leflunomide and cyclosporine are sometimes used in progressive MS, but they are teratogenic and carcinogenic.

For this reasons scientists are now involved in the search of new therapeutic agents capable of defusing destructive immune cells, leaving the rest of the body's protective immune system intact.

So far, such studies indicate a more clear role carried on by T lymphocytes. T lymphocytes are able to distinguish between substances belonging to the body (self) and substances that are foreign. They recognize one specific antigen (normally a peptide fragment) through a protein protruding from the T cell surface, the T cell receptor (TCR), only when this fragment is associated with a molecule of the major histocompatibility complex (MHC), localized on the surface of specialized antigen presenting cells. The trimolecular complex formed (MHC-peptide-TCR) seems to be responsible for activation of an autoreactive T cell clone, which releases proteins as interferon γ and the tumor necrosis factor, that are responsible for MS demyelinating process.

It is known that T cell receptor can recognize modified ligands and respond in a variety of ways through different mechanisms as TCR antagonism (De Magistris et al., "Cell", 68, 625-634, 1992), T cell tolerance or T cell anergy (Schwartz, "Science", 248, 1349-1356, 1990; Sloan-Lancaster et al., "Nature", 363, 156-159, 1993).

Apparently that anergy can be induced by synthetic peptides corresponding to the major immunodominant T cell determinants of native protein antigens, which are able to induce T cell unresponsiveness to themselves (Vidard et al., "Proc. Natl. Acad. Sci. USA" 92, 2259-2262, 1995). Anergic lymphocytes are incapable of producing IL-2 on restimulation with antigen (Schwartz, "Science", 248, 1349-1356, 1990).

For example, substitution of a proline (96→Pro) with an alanine in the encephalitogenic peptide epitope 87-99 Val-His-Phe-Phe-Lys-Ile-

Val-Thr-Pro-Arg-Thr-Pro from human Myelin Basic Protein (hMBP), caused T cell hyporesponsiveness, with a reduced ability to induce a proliferative response in a T cell clone specific for MBP. However, such peptide is still capable of causing encephalomyelitis in a susceptible mouse strain (Broke, S., et al., "Nature", 379, 343-346, 1996).

This approach did not lead to clinically useful results since altered peptides which are able to specifically act on the autoreactive T cell subset, may cause in certain conditions also the disease.

For these reasons, it is still felt the need of a peptide capable of inducing a state of unresponsiveness (anergy) of autoreactive T lymphocytes in susceptible people or in individuals affected by MS without inducing an autoreactive response.

Now it has been found that these properties are owned by a peptide compound selected from the group comprising:

R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)

R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)

R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)

R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)

where

R is H- or $COCH_3$, R' is COOH or $CONH_2$ and

each amino acid has L or D configuration.

It is therefore a first object of the present invention to provide a peptide compound selected from the group comprising:

R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)

R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)

R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)

R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)

where

R is H- or $COCH_3$, R' is COOH or $CONH_2$ and

each amino acid has L or D configuration.

The peptide compounds of this invention may be prepared according to conventional methods used in peptide chemistry for suitable protection of amino acids, coupling of protected amino acids and removal of protective groups.

Preferably said peptides are prepared according to solid-phase synthesis techniques, which comprise the following steps:

- coupling, by means of a suitable reagent, of the first C-terminal amino acid protected both at the alpha amino group and amino group, if any, at the side chain to a suitable support for solid phase synthesis;
- removal of the alpha amino protective by means of suitable reagent;
- coupling of the second amino acid, starting from the C-terminal end, protected both at the alpha amino group and at the side chain amino group, if any;
- removal of the protective group at the alpha amino group and performance of the coupling and deprotection steps until completion of the couplings of all the amino acids encompassed in the peptide sequence up to the N-terminal residue;

– removal of the remaining side chain protective groups and detachment of the assembled peptide from the support for solid phase synthesis.

These techniques are widely described in the literature and are well known to the person skilled in the art (Atherton & Sheppard, 1989, Solid Phase Peptide Synthesis, IRL Press, Oxford, UK).

It is therefore a second object of the present invention to provide a pharmaceutical composition, which comprises a therapeutically effective dose of a peptide selected from the group comprising:

R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)

R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)

R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)

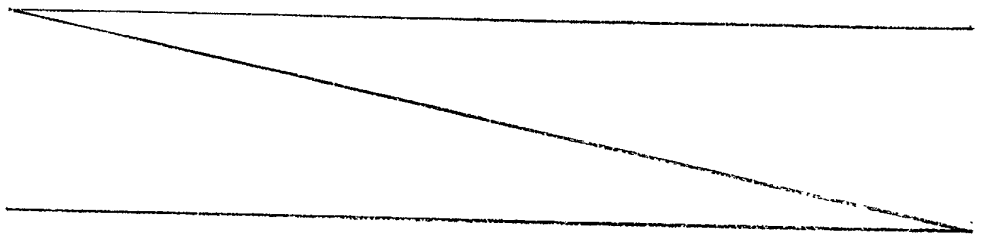
R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)

where

R, R', the amino acid configuration have the above mentioned meanings, and

at least a pharmaceutically acceptable inert ingredient.

It is still another object of this invention to provide a method of treating a patient suffering from Multiple Sclerosis, said method comprising administering to a patient in need thereof an effective amount of a peptide compound selected from the group comprising:



R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)

R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)

R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)

R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)

where

R, R' and the amino acid configuration have the above mentioned meanings.

Preferably, the pharmaceutical compositions according to this invention are prepared in a suitable dosage form comprising an effective dose of at least a peptide compound of formula (I) to (IV) (SEQ ID NOS: 1-4) and at least a pharmaceutically acceptable inert ingredient.

Examples of suitable administration routes are the inhalation or oral route; as they are known to induce a state of immunologic hyporesponsiveness (oral tolerance). (Grainstein, et al., "Chem. Immunol.", 58, 259-290, 1994).

Typical examples of suitable dosage forms are pills, capsules, sugar coated pills, granules, solutions and syrups for oral administration, unguents and plasters for topic administration; suppositories for rectal administration and sterile solutions for injectable, inhalation and ophthalmic administration.

The dosage forms may also contain other conventional ingredients like preservatives, stabilizers, surface-active agents, buffers, salts to regulate the osmotic pressure, emulsifying agents, sweeteners, dyes, flavors and so on.

When required by particular therapies, the pharmaceutical composition of this invention may contain other active pharmacological ingredients whose concomitant administration is therapeutically useful.

Typically the amount of the peptide compound of this invention in the pharmaceutical composition of the invention will be such that it insures an administration level of from 1 to 100 mg/Kg/day.

The amount of a peptide compound of formula from (I) to (IV) (SEQ ID NOS: 1-4) in a pharmaceutical composition of this invention may vary in a rather wide range depending on known factors such as, for example, the severity of the disease, the body weight of the patient, the dosage form, the chosen route of the administration, the number of dosage forms administrated daily and the specific efficacy of the chosen peptide compound of formula (I) to (IV) (SEQ ID NOS: 1-4).

The dosage forms of the pharmaceutical composition of this invention can be prepared according to techniques which are known to the pharmaceutical chemist and comprise procedures such as mixing, granulation, compression, dissolution, sterilization and so on.

As described in detailed below (Assay 1), the biological activity of the peptide compounds of this invention has been evaluated by *in vivo* studies on a susceptible strain of mice suitable to develop experimental autoimmune encephalomyelitis (EAE), which represents a murine model of MS.

Indeed, both MS and EAE are a $CD4^+$ T cell mediated disease and can be induced by simply immunization of mice with MBP or with one of its encephalitogenic peptide fragments. EAE, as MS, is characterized by invasion of the central nervous system by T lymphocytes, resulting in demyelination and acute, chronic or chronic relapsing paralysis.

Assay 1 shows that peptide compound of the present invention are able to delay the onset of clinical signs of the disease, diminishing the severity and increasing the survival rate of treated mice.

The present invention is further described by the following Examples and Assays which are for illustrative purposes only and should not be construed as a limitation of the invention.

EXAMPLE 1

Peptide Compounds of formula (I) (SEQ ID NO: 1), (II) (SEQ ID NO: 2) and Comparison Peptide Compound P81-100 (SEQ ID NO: 5) (corresponding to 81-100 sequence of the encephalitogenic mouse MBP epitope)

The peptide compounds were synthesized by solid phase synthesis following the Fmoc/DCC/HOBt methodology (Bodanszky, "Principles of peptide synthesis", Springer-Verlag, New York, 1984) on a fully automated Model 431A Applied Biosystem peptide synthesizer, software version 1.2, following the manufacturer instruction. The synthesis was carried out on the acid labile resin (Wang resin) (NOVABIOCHEM, Laufelfinger, CH) for peptide synthesis pre-derivatized with Fmoc-Glycine (NOVABIOCHEM, Laufelfinger, CH No. Cat. 04-12-2010, 0.1 mmole) for the synthesis of the peptide compound of formula (I) (SEQ ID NO: 1) and pre-derivatized with Fmoc-glutamine (trytil) (NOVABIOCHEM, Laufelfingen, CH No. Cat. 04-12-2036, 0.1 mmol) for the synthesis of the peptide compound of formula (II) (SEQ ID NO: 2) and peptide compound P81-100 (SEQ ID NO: 5). The N-terminal Fmoc protected amino group has been removed in the first synthesis cycle by treatment with 3 mL of 20 % pyridine in N-methyl-pyrrolidone (Merck, Darmstadt, Germany) for 14 minutes at room temperature under agitation. The deprotected resin has been then washed 5 times with N-methyl-pyrrolidone (2.5 mL) for 9 minutes at room temperature. In the mean time, the second amino acid to be added (1 mmole) has been activated at the carboxyl end by treatment with 1 mL of 1 M HOBT in N-methyl-pyrrolidone and 1 mL of 1 M dicyclohexylcarbodiimide (DCC) in N-methyl-pyrrolidone. The activated amino acid has been then

incubated with the resin for 51 minutes under agitation. The resin has been then washed four times with 2 mL of N-methyl-pyrrolidone for 0.5 minutes. At this point the resin has been deprotected with piperidine and subjected to a subsequent coupling step with the next amino acid. This procedure has been repeated in a sequential manner for all the amino acids protected as indicated below:

Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Lys(Boc)-OH, Fmoc-Pro-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Phe-OH, Fmoc-Ile-OH. After completion of the synthesis, and after removal of the N-terminal Fmoc group with piperidine, the resin has been washed with methanol, dichloromethane, and again with methanol (MERCK, Darmstadt, Germany) and accurately dried under vacuum for 12 hours. Then the peptidic material has been detached from the resin by treatment of 200 mg of the resin with 5 mL of a mixture of trifluoroacetic acid (Pierce, Rockford, IL, USA), phenol (Sigma-Aldrich, Milan, Italy) thioanisol (Sigma-Aldrich, Milan, Italy) water (Sigma-Aldrich, Milan, Italy) and ethandithiol (Sigma-Aldrich, Milan, Italy) in the ratio 83:6:5:4:2 at room temperature for 3.5 hours. The mixture has been then filtered on a sintered glass funnel and the peptidic material precipitated by the addition of 10 mL of cold ethyl ether. The peptidic material has been obtained by centrifugation and the pellet has been dissolved in 25 mL of a mixture of water, acetonitrile (Merck, Darmstadt, Germany), and trifluoroacetic acid in the ratio 90:10:0.1, and purified by semipreparative RP-HPLC on a Aquapore column (Applied Biosystems, Foster City, CA, USA). Material corresponding to the main peak has been collected, frozen, and lyophilized. The peptide compounds were characterized by analytical RP-HPLC, amino acid analysis, and by determination of peptide resins were dried overnight under vacuum. Peptides compounds were cleaved from resin using

TFA/phenol/thioanisole/H_2O/ethanedithiol 83:6:5:4:2 (5 mL/200 mg of resin), and incubating at room temperature for 3.5 h. The mixtures were then filtered and the peptidic material was precipitated by adding 10 mL of cold ethyl ether. After lyophilization the products were dissolved in H_2O/CH_3CN/TFA 90:10:0.1 and purified by semipreparative RP-HPLC. After purification the peptide were characterized by analytical HPLC, amino acid analysis and TOF-MALDI mass spectrometry on a KRATOS Kompact MALDI III (KRATOS Analyticals, Manchester, UK) using sinapinic acid as the matrix (Sigma-Aldrich, Milan, Italy).

The following peptide compounds were thus prepared:

Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly (I) (SEQ ID NO: 1)

Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln (II) (SEQ ID NO: 2)

Asn-Pro-Val-Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro-Pro-Pro-Ser-Gln (P81-100) (SEQ ID NO: 5)

In the same way the following peptides were also prepared:

Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly (III) (SEQ ID NO: 3)

Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn (IV) (SEQ ID NO: 4)

Assay 1

In vivo activity of the peptide compound of formula (I) (SEQ ID NO: 1) and (II) (SEQ ID NO: 2)

The *in vivo* activity of the peptide compounds of formula (I) and (II) (SEQ ID NOS: 1-2), were evaluated on four groups of SJL female mice, used at the age of 6-15 weeks. This strain of mice has been genetically selected for its ability to develop experimental allergic encephalitis (EAE).

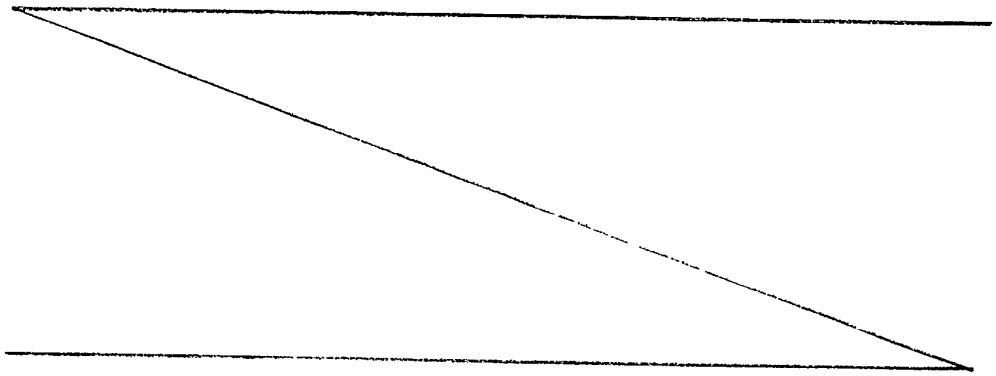
Two groups of mice have been immunized intraperitoneally with the peptide compound of formula (I) and (II) (SEQ ID NOS: 1-2) (300 nmoles), respectively, dissolved in 0.05 ml of physiological solution and then emulsified with 0.05 ml of incomplete Freund's adjuvant (IFA) (Sigma, Italy).

As positive and negative control, P81-100 (SEQ ID NO: 5) and IFA alone were used respectively.

After two weeks EAE was induced in all groups by challenge with 300 nmoles of P81-100 (SEQ ID NO: 5) dissolved in 0.1 ml of physiological solution and then emulsified with 0.1 ml of complete Freund's adjuvant (CFA) (Sigma, Italy).

Pertussis toxin (200 ng) (Fluka Chemie AG, Switzerland) was injected intravenously at the time of immunization and 2 days later. The role of pertussis toxin is to enhance the migration of autoreactive lymphocytes through the encephalic barrier (Bernard, et al.,"J. Immunol." 114, 1537-1540, 1975).

Mice were observed daily beginning at day 9 up to 60 for clinical signs of EAE and were graded according to the following clinical scale: 1, limp tail; 2, partial hind limp paralysis; 3, complete hind limp paralysis; 4, hind and front limp paralysis; 5, moribund (Broke S. et al., "Nature", 379, 343-346, 1996). Grade 5 mice were euthanized.



The results are shown in the Table 1.

Peptide (nmol)	Incidence of EAE *	Day of on-set**	Severity***
P81-100 (300)	2/17	33±4.24	1.0±0
IFA	4/11	18.0±2.0	3.2±1.3
Peptide I	0/17	-	-
Peptide II	2/17	57.5±3.53	3±2.82

* Incidence of EAE is expressed as number of mice with clinical EAE/number of mice immunized.

** The day of onset is expressed as mean day of onset ± SD (Standard Deviation).

*** The severity is expressed as mean severity of sick mice ± SD.

Data from Table I shows that mice treated with the peptide compound of formula (I) (SEQ ID NO: 1) didn't develop EAE. Mice treated with the peptide compound of formula (II) (SEQ ID NO: 2) developed clinical signs of EAE but with a reduced incidence and an increase of the delay of the onset compared to the positive control (IFA group).

CLAIMS

1. A peptide selected from the group comprising:
R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)
R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)
R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)
R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)
where
R is H- or $COCH_3$, R' is COOH or $CONH_2$ and
each amino acid has L or D configuration.

2. A pharmaceutical composition comprising an effective dose of at least a peptide compound selected from the group comprising:
R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)
R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)
R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)
R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)
where
R is H- or $COCH_3$, R' is COOH or $CONH_2$, each amino acid has L or D configuration, and
at least a pharmaceutical acceptable inert ingredient.

3. A method of treating a patient suffering from Multiple Sclerosis, said method comprising administering to a patient in need thereof an ef-

fective amount of a peptide compound selected from the group comprising:

R-Asn-Gly-Val-Gly-His-Gly-Phe-Gly-Asn-Gly-Val-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Ser-Gly-R' (I) (SEQ ID NO: 1)

R-Gly-Pro-Gly-Val-Gly-Phe-Gly-Lys-Gly-Ile-Gly-Thr-Gly-Arg-Gly-Pro-Gly-Pro-Gly-Gln-R' (II) (SEQ ID NO: 2)

R-Gln-Gly-Pro-Gly-Pro-Gly-Arg-Gly-Thr-Gly-Ile-Gly-Lys-Gly-Phe-Gly-Val-Gly-Pro-Gly-R', and (III) (SEQ ID NO: 3)

R-Gly-Ser-Gly-Pro-Gly-Thr-Gly-Pro-Gly-Val-Gly-Asn-Gly-Gly-Phe-Gly-His-Gly-Val-Gly-Asn-R' (IV) (SEQ ID NO: 4)

where

R is H- or $COCH_3$, and R' is COOH or $CONH_2$ and

each amino acid has L or D configuration.

SEQUENCE LISTING

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epitope

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Pro Pro Ser Gln
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COPY

Declaration and Power of Attorney for Patent Application
Dichiarazione e procura ai fini della domanda di brevetto

Italian Language Declaration

Il sottoscritto inventore dichiara che:

As a below named inventor, I hereby declare that:

La propria residenza, recapito postale e cittadinanza corrispondono a quanto indicato in calce, sotto la propria firma.

My residence, post office address and citizenship are as stated next to my name.

Ritiene di essere il primo ed unico inventore originale (se viene elencato in calce un solo nominativo) o il coinventore primo ed originale (se è elencato più di un nominativo) del oggetto rivendicato e per il quale il sottoscritto presenta domanda di brevetto. La invenzione in questione è chiamata.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

"Peptides useful in treating Multiple Sclerosis and a pharmaceutical composition comprising the same"

e la sua descrizione è allegata alla presente Dichiarazione a meno:

the specification of which:

☐ è qui allegato

☐ is attached hereto.

☐ Il _____ è stata depositata una domanda di brevetto statunitense numero o una domanda di brevetto internazionale PCT numero _____ che è stata modificata il _____ (se applicabile).

☐ was filed on March 29, 1999 as United States Application Number or PCT International Application Number PCT/EP99/02268 _____ and was amended on _____ (if applicable).

Il sottoscritto dichiara in oltre di aver letto e compreso il contenuto della descrizione identificata in precedenza, rivendicazioni comprese, come modificati dall'eventuale modifica summenzionata.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

Il sottoscritto riconosce l' obbligo di rivelare informazioni essenziali ai fini della determinazione della brevettabilità ai sensi del Titolo 37, Codice dei Regolamenti Federali, § 1.56.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

Italian Language Declaration

Il sottoscritto rivendica con la presente la priorità prevista dal Titolo 35, Codice degli Stati Uniti, § 119(e)-(d) o § 365(b) in relazione a qualsiasi domanda o domande estere di brevetto o certificato di inventore, o dal Titolo 35, § 365(a) degli stessi Codice in relazione a qualsiasi domanda internazionale PCT nella quale è designato almeno un paese diverso dagli Stati Uniti, i suddetti domande e certifcati essendo elencati sotto, e, spuntando les seguenti caselle, ha anche identificato sotto qualsiasi domanda estera di brevetto o certificato di inventore, o domanda internazionale PCT, la cui data di deposito preceda quella dalla domanda per la quale è rivendicata la priorità.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)
(Domande Estere Anteriori)

(Number) (Numero)	(Country) (Nazione)	(Day/Month/Year Filed) (Giorno/Mese/Anno di deposito)	Priority claimed Diritto di priorità rivendicato ☐ Yes Sì	☐ No No
(Number) (Numero)	(Country) (Nazione)	(Day/Month/Year Filed) (Giorno/Mese/Anno di deposito)	☐ Yes Sì	☐ No No

Il sottoscritto rivendica con la presente i benefici previsti dal Titolo 35, Codici degli Stati Uniti, § 119(e), in relazione a qualsiasi domanda o domande provvisorie degli Stati Uniti elencate sotto.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)

Il sottoscritto rivendica con la presente i benefici previsti dal Titolo 35, Codice degli Stati Uniti, § 120, in relazione a qualsiasi domanda o domande statunitensi, o dal Titolo 35, § 365(c) degli stessi Codice in relazione a qualsiasi domanda internazionale PCT nella quale sono designati gli Stati Uniti, i suddette domande essendo elencate sotto e, nella misura in cui l'oggetto di ciascuna rivendicazione di questa domanda non sia stato esposto nella domanda statunitense o internazionale PCT anteriore nel modo previsto dal primo paragrafo del Titolo 35, Codice degli Stati Uniti, § 112, riconosce l'obbligo di rivelare informazioni essenziali ai fini della determinazione della brevettabilità ai sensi del Titolo 37, Codici dei Regolamenti Federali, § 1.56, le quali diventino disponibili durante il periodo compreso tra la data di deposito della domanda anteriore e la data di deposito nazionale o internazionale PCT della presente domanda.

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Status) (patented, pending, abandoned) (Stato) (concessione di brevetto, in corso di esame, abbandono)
(Application No.) (Nº della domanda)	(Filing Date) (Data di deposito)	(Status) (patented, pending, abandoned) (Stato) (concessione di brevetto, in corso di esame, abbandono)

Con la presente, il sottoscritto dichiara veritiere tutte le affermazioni contenute in questa domanda in relazione alle proprie conoscenze e di ritenere vere tutte le affermazioni o informazioni presentate. Dichiara inoltre che tali asserzioni sono state espresse nella piena consapevolezza che le dichiarazioni intenzionalmente false sono punibili con una poulte, l' incarcerazione o entrambe, ai sensi della Sezione 1001 del Titolo 18 del Codice degli Stati Uniti e che tali dichiarazioni entenzionalmente false possono mettere a repenfaglio la validità della domanda o di qualsiasi brevetto ruasciato in merito.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Italian Language Declaration

PROCURA: Il sootscritto inventore nomina con la presente il seguente avvocato o avvocati e/o agente o agenti al fine di istruire questa pratica e di condurre tutte le operazione ad essa pertinenti presso l'Ufficio dei Brevetti e Marchi di Fabbrica: (Elencare il nome ed il numero di matricola).

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

022850

Inviare le corrispendenza a:

Send Correspondence to:

022850

Telefonare a:
(Nome e numero telefonico)

Direct Telephone calls to: *(name and telephone number)*

(703) 413-3000

Nome e cognome dell'unico o del primo inventore 1-00 MARINO Maria	Full name of sole or first inventor
Firma dell'inventore Data	Inventor's signature Date Sept. 26, 2001
Residenza CASERTA - Italy ITX	Residence
Cittadinanza Italian ✓	Citizenship
Recapito postale Via Rossini, 5 81100 CASERTA - Italy	Post Office Address
Nome e cognome dell'eventuale secondo coinventore 2-00 IPPOLITO Agostino	Full name of second joint inventor, if any
Firma del secondo coinventore Data	Second inventor's signature Date Sept. 26, 2001
Residenza NAPOLI - Italy ITX	Residence
Cittadinanza Italian ✓	Citizenship
Recapito postale Via V. Janfolla II traversa, 3 - 80145 NAPOLI - Italy	Post Office Address

(Fornire le stesse informazioni e le firme del terzo e degli ulteriori coinventori.)

(Supply similar information and signature for third and subsequent joint inventors

Italian Language Declaration

Nome per intero di un eventuale terzo co-inventore FASSINA Giorgio	Full name of third joint inventor, if any
Firma del Terzo Inventore Data	Third inventor's signature Date Sept. 26, 2001
Residenza MILANO - Italy ITX	Residence
Cittadinanza Italian ✓	Citizenship
Recapito postale Via Bassini, 49 20133 MILANO - Italy	Post Office Address
Nome per intero di eventuale quarto co-inventore	Full name of fourth joint inventor, if any
Firma Quarto Inventore Data	Fourth inventor's signature Date
Residenza	Residence
Cittadinanza	Citizenship
Recapito postale	Post Office Address
Nome per intero di un eventuale quinto co-inventore	Full name of fifth joint inventor, if any
Firma Quinto Inventore Data	Fifth inventor's signature Date
Residenza	Residence
Cittadinanza	Citizenship
Recapito postale	Post Office Address
Nome per intero di un eventuale sesto co-inventore	Full name of sixth joint inventor, if any
Firma del Sesto Inventore Data	Sixth inventor's signature Date
Residenza	Residence
Cittadinanza	Citizenship
Recapito postale	Post Office Address

(Si prega di fornire simili informazioni e firme peril terzo e gli eventuali ulteriori co-inventori.)

(Supply similar information and signature for third and subsequent joint inventors.)

SEQUENCE LISTING

<110> MARINO, MARIA

IPPOLITO, AGOSTINO

FASSINA, GIORGIO

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